

# REPORT DOCUMENTATION PAGE

Form Approved  
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this

1. AGENCY USE ONLY (Leave blank)

2. REPORT DATE  
10/10/00

3. REPORT TYPE AND DATES COVERED  
Final Report 5/1/93 - 3/31/00

4. TITLE AND SUBTITLE

Biological Rules and Mechanisms Governing the Nanofabrication of Highly Regular Mineralized Microlaminate Composites

5. FUNDING NUMBERS

N00014-93-1-0584

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8. PERFORMING ORGANIZATION  
REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Office of Naval Research  
800 N. Quincy St.  
Arlington, VA 22217-5000

10. SPONSORING / MONITORING  
AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

We purified and characterized the proteins from the microlaminate abalone shell (a natural high-performance armor with fracture-toughness 3,000-fold greater than that of its mineral component alone) and "flat pearl" and from sponge and diatom biosilicas. We then cloned and sequenced the cDNAs encoding these proteins, and used the resulting structural information, in concert with site-directed mutagenesis (genetic engineering) and real-time atomic force microscopy and other advanced imaging techniques to reveal the fundamental mechanisms by which the proteins control the mineral nucleation, growth and nanocomposite structures based on both silicon and calcium. Advanced imaging with AFM, XRD, SICM, EDAXAX and NMR was used to resolve the mechanisms controlling synthesis and shape in biomineralization and biomimetic synthesis, and details of the structure-directing organic-inorganic interfacial interactions. We then used this information to make useful new mineral-organic composite materials in vitro.

14. SUBJECT TERMS

biomineralization, biomimetic, nanofabrication, microlaminate, composite, materials, shell, mineral, protein

15. NUMBER OF PAGES

4

16. PRICE CODE

17. SECURITY CLASSIFICATION  
OF REPORT  
Unclassified

18. SECURITY CLASSIFICATION  
OF THIS PAGE  
Unclassified

19. SECURITY CLASSIFICATION  
OF ABSTRACT  
Unclassified

20. LIMITATION OF ABSTRACT  
--- Unlimited

DTIC QUALITY INSPECTED 4

20001107 118

## **FINAL REPORT**

**Grant Number:** N00014-93-1-0584

**Principal Investigators:** Daniel E. Morse, G. D. Stucky and P. K. Hansma

**Institution:** University of California at Santa Barbara

**Grant Title:** "Biological Rules and Mechanisms Governing the Nanofabrication of Highly Regular Mineralized Microlaminate Composites"

**Award Period:** 01 May, 1993 - 31 March, 2000

**OBJECTIVE:** To identify and characterize the proteins, genes and molecular mechanisms governing the biological nanofabrication of mineralized composite materials, and harness these to develop new and environmentally benign routes to the synthesis of high-performance composites.

**APPROACH:** We purified and characterized the proteins from the microlaminate abalone shell (a natural high-performance armor with fracture-toughness 3,000-fold greater than that of its mineral component alone) and "flat pearl" and from sponge and diatom biosilicas. We then cloned and sequenced the cDNAs encoding these proteins, and used the resulting structural information, in concert with site-directed mutagenesis (genetic engineering) and real-time atomic force microscopy and other advanced imaging techniques to reveal the fundamental mechanisms by which the proteins control the mineral nucleation, growth and nanocomposite structures based on both silicon and calcium. Advanced imaging with AFM, XRD, SICM, EDAX and NMR was used to resolve the mechanisms controlling synthesis and shape in biomineralization and biomimetic synthesis, and details of the structure-directing organic-inorganic interfacial interactions. We then used this information to make useful new mineral-organic composite materials in vitro.

**ACCOMPLISHMENTS:** We discovered that the origin of both the exquisite nanostructural control and of the associated high-performance capabilities of these biomaterials lies in the unique mechanisms of their synthesis. In addition to the mechanisms of templating, epitaxy and polymer control of crystal nucleation and growth that were anticipated at the start of these studies, unanticipated new discoveries of self-assembling molecular stencils, novel self-healing energy-dispersive elastomers and structure-directing catalysts help explain the molecular mechanisms underlying the fabrication and performance of biologically produced mineral-organic composites. AFM analyses revealed that the microlaminate structure of nacre is organized over macroscopic dimensions by continuous growth of atomically coherent columns of protein-oriented aragonite through pores in a multilayered network of sheets of biopolymer that also are trilaminate. Stencil-like nanopores in these interlamellar matrix sheets guide the growth of the crystal columns from one layer to the next. The stochastic spacing of these nanopores apparently determines the lateral offset between successive microlaminae, thus generating the interdigitating brickwork that contributes to the material's remarkable strength. The sequence of Lustrin A cloned from this matrix reveals a strikingly repetitive,

alternating modular structure that helps explain its properties as an energy-dissipating elastomeric adhesive linker. Use of the atomic force microscope enabled us to measure the forces required to open successive domains in this modular elastomer, and to analyze its hysteretic "self-healing recovery. We thus discovered how the unique molecular structure and properties of Lustrin A contribute to the resiliency of the mineral-organic microlaminate composite. We discovered that silica needles are formed by a unique class of structure-directing, catalytically active proteins; we then used site-directed mutagenesis to identify the structural determinants of this catalysis.

These counterintuitive new findings, overturning the previous paradigms concerning the mechanisms of biological fabrication with both calcium and silicon, now are leading to the development of new low-temperature biomimetic routes to the synthesis of advanced mineral-organic composites. Use of the purified polyanionic proteins from the abalone shell allowed us to abruptly and sequentially switch crystallographic phase with stereospecific precision and fidelity, producing multiphase composites with micron-scale domains. We also used these proteins to produce crystalline thin-films of isostructural metal carbonates with useful optical, magnetic and electronic properties. Translating the molecular mechanisms of biosilica synthesis that we discovered to "biomimetic" chemical routes to new materials, we accomplished the first synthesis of mirrorless nanoscale lasers based on our biomimetically organized mesoporous silica channels.

**CONCLUSIONS:** Our principal conclusions are (1) that biology has evolved unique mechanisms for the synthesis and nanostructural organization of high-performance mineral-organic composite materials; (2) that these can be resolved through the use of biotechnology; and (3) that these mechanisms can then be harnessed to develop new low-temperature routes to high-performance magnetic, electronic and structural materials based on silicon and various metals.

**SIGNIFICANCE:**

This work represents the first purification, characterization and sequencing of proteins occluded within a biosilica, and the first discovery that such proteins and their cloned DNA can be used to chemically and spatially direct the polymerization of siloxanes in vitro. In addition to revealing the underlying molecular biological mechanism controlling the nanofabrication of silicon-based materials under mild physiological conditions, our findings demonstrate that this mechanism can be harnessed for the development of environmentally benign new routes to the synthesis of patterned silicon-based materials from a variety of tailored feedstocks.

Our studies of both the biosilica and calcium carbonate composites reveal the same fundamental lesson despite the vast differences in the chemistry and molecular biology of these two systems: In both, Nature uses two very different mechanisms to control mineral-composite synthesis and shape on the atomic scale and at the mesoscale level. Harnessing these mechanisms biomimetically, we have produced controlled silica architectures with control over porosity dimensions over length-scales ranging from angstroms to centimeters, with corresponding control of mechanical and optical properties yielding the first mirrorless nanoscale lasers. These, in turn, represent one of the first steps toward production of integrated optical circuits, analogous to the integrated electronic circuits that are in ubiquitous use today.

#### **PATENTS:**

- "Inorganic/Block Copolymer-Dye Composites and Dye-Doped Mesoporous Materials for Optical and Sensing Applications," Gernot Wirnsberger, Brian J. Scott, Nicholas A. Melosh, Howard C. Huang, Peidong Yang, Bradley F. Chmelka, Galen D. Stucky. 2000
- "Bifunctional Catalysts for Production of Silica, Glass, Silicones and Polymetallo-oxanes at Low Temperatures and Neutral pH." Daniel E. Morse, Galen Stucky and Yan Zhou. 1999.

#### **AWARD INFORMATION:**

- Co-PI Prof. Paul Hansma was honored by the American Physical Assocn. as the Outstanding Biological Physicist of the Year, 1999.
- Each of the 3 co-PIs was honored as Keynote Speaker at multiple International Symposia.

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